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Anthracnose disease of Painted evergreen [*Aglaonema crispum* (Pitcher & Manda) Nicolson] caused by *Colletotrichum gloeosporioides* from West Bengal

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Abstract
Painted evergreen or Chinese evergreen [*Aglaonema crispum* (Pitcher & Manda) Nicolson] is an economically important foliage ornamental plant grown in the garden of Agri-Horticultural Society of India at Kolkata, West Bengal. These were found to be attacked severely by anthracnose disease leading to devastating foliar damage. The leaves showed characteristic blighting like symptoms with light brown to grey necrotic areas bordered by dark brown wavy margins. On the necrotic areas, black, erumpent, dot like acervuli, 320.7 – 540.1µ wide appeared to be arranged in the form of concentric zonations on the upper surface of the leaf. Setae were numerous, 1 - 2 septate, 126.0 – 176.3 x 3.5 – 6.8 µ in size with pointed tips. Conidia were hyaline, single celled, cylindrical to short rod shaped with rounded ends and 18.5 – 24.8 x 2.7 – 4.4µ in size. On the peptone agar medium (PAM) identified as ideal medium for acervuli production and sporulation, acervuli were pale brown to black, 426.5 - 920.5 µ in size. Setae were numerous, black to dark brown, 1 – 3 septate, unbranched, 198.5 – 245.1 x 6.0 – 7.8 µ in size with pointed tips. Conidia were hyaline, 1-celled, smooth walled, eguttulate, cylindrical with rounded ends measuring 23.7– 28.5 x 3.0 – 5.0 µ in size. Pathogenicity test of the isolated fungus had been established under laboratory condition following detached leaf technique. On comparison of the isolated fungus with *Gloeosporium graffi* and *Colletotrichum dematium* which were known to attack the host, it was found that acervuli and conidial characteristics of causal fungal pathogen of the present study differed from *Gloeosporium* due to the presence of setae and also from *Colletotrichum dematium* due dissimilarity in spore shape and size but exhibited gross similarity with *Colletotrichum gloeosporioides*. So, the causal fungus of presently described anthracnose disease of *Aglaonema crispum* is being proposed as *Colletotrichum gloeosporioides*.

Keywords: Chinese evergreen, *Aglaonema*, Painted evergreen, anthracnose, ornamental diseases.

Introduction
Aglaonema is a genus of flowering plants in the arum family, Araceae. They are native to tropical and subtropical regions of Asia and New Guinea and are commonly known as Chinese evergreens. It is a genus of about 25 species among them, *Aglaonema crispum* is an erect, evergreen perennial plant and are known under the following names: "drop-tongue", "painted drop-tongue", "painted evergreen" (English); "Kolbenfaden" (German).Their foliage is elliptical with dark green margins and ample silvery green cast, these striking ornamental foliage, impart economic importance to the plant. They thrive well under shade and water sparse conditions. *Aglaonema* aid in removal of harmful toxins, thus listed among the top performers in NASA's clean air study. It has been reported from different parts of the world including India that *Aglaonema* is approximately attacked by 5 fungal, 3 bacterial and few viral and nematode diseases (Table-1).Among them anthracnose disease severely infects the foliage and rapidly destroys the whole plant, making the plant less marketable by reducing their aesthetic value.

Literature suggests that the genus *Aglaonema* suffers from anthracnose caused by *Gloeosporium graffi* and *Colletotrichum* spp. The anthracnose of *Aglaonema costatum,* caused by *Gloeosporium graffi*, produced large, more or less circular to elliptical, scattered spots, up to 1.5 cm. across, buff at the centre with salmon-coloured margins. The spots later became necrotic with numerous acervuli which are dark, epiphyllous, mostly on central regions, punctiform, discoid, sub-cuticular to erumpent. Conidia were hyaline, 1-celled, smooth, ellipsoid, guttulate, 11 - 13.5 µ x 3.5µ. It was known to attack *A. simplex* also (*c.f* Sohi, 1990). Gu and Zhu (1994) reported the occurrence of *Colletotrichum gloeosporioides [Glomerella cingulata]* from China as the cause of anthracnose of *A. modestum.* They observed that spore germination was optimal at 25 ^{0}C and opt. hyphal growth occurred at 20 - 25 ^{0}C and pH 5 – 6.

The disease was also observed on Chinese evergreen, *Aglaonema blume (A. pseudobracteatum, A. pictum, A. treubii)* plants grown in protected ornamental crops of Corrientes, Chaco and Formosa provinces in the northeast of Argentina. Affected leaves showed grayish, irregular, extensive spots, with slightly raised dark edges. Black fruiting bodies could be observed in their centers. The pathogens were identified in the leaves of disease affected plants as *Colletotrichum dematium* (Pers. ex Fr.) Grove, was the new pathogen in *Aglaonema* spp. It was the first report of *Aglaonema* anthracnose disease in Argentina. (Cabrera and Alvarez, 2001)

Table I: Disease spectrum of *Aglaonema*

Disease	Causal organism	Reference
Fungal		
Anthracnose	*Gloeosporium graffi* *Colletotrichum sp.*	- *c.f* Sohi, 1990 -From Argentina by Cabrera and Alvarez, (2001) -From China by Gu and Zhu (1994)
Phytophthora blight	*P. meadii* *P. nicotianae var. parasitica*	From Taiwan (Ann, 1992)
Collar rot and foliar blight disease	*Fusarium subglutinans [Gibberella fujikuroi var. subglutinans]*	From Hawaii, USA by Uchida and Aragaki (1994)
Sphaeropsis leaf blight	*Sphaeropsis modestum sp. nov*	From Nauni, Solan, India by Gupta and Sunita (1996)
BACTERIAL		
Burkholderia leaf spot	*Burkholderia gladioli on Aglaonema commutatum*	From Argentina by Alippi and Lopez, (2009)
Xanthomonas leaf spot	*Xanthomonas sp. on Aglaonema commutatuma*	From Assam by Madhusmita *et al.*, 1999
Erwinia blight	*Erwinia herbicola on A. marantifolium and A. treubi*	From Venezuela by Contreras *et al*. (1994) and Arias *et al.* (1998)
Virus	Dasheen mosaic virus	From Cairo, Egypt (Fawzy, 1996) and from Taiwan, China. (Liang *et al*., 1994).
Nematodes	*Pratylenchus coffeae* *Helicotylenchus californicum* *Aphelenchus spp.* *Tylenchus spp.* and *Scutellonema spp.*	From Costa Rica (Marban and Flores, 1993)

Materials and Methods

A detailed study on the disease along with its causal agent had been conducted during present investigation. The diseased leaf sample of *Aglaonema crispum* grown inside and outside of the greenhouse of Agri-Horticultural society of India, Kolkata, West Bengal(located at 22˚53'N latitude and 88°33' E longitude) were collected in brown paper packets and detailed *in situ* description of symptoms were done. The severity of the foliage damage caused was assessed using the 0 - 6 scale (Table II). The percent damage caused was recorded by visual observation and scoring the plants in the greenhouse.

Table II: Descriptions of 0 – 6 disease scoring scale with respective reaction categories

Scale	Description	Reaction categories
0	No infection or 0% infection	Immune
1	1-5% leaf area /length covered by disease	Highly resistant
2	6-10% leaf area /length covered by disease	Resistant
3	11-25% leaf area /length covered by disease	Moderately resistant
4	26-50% leaf area /length covered by disease	Moderately susceptible
5	51-75% leaf area /length covered by disease	Susceptible
6	76-100% leaf area /length covered by disease	Highly susceptible

Samples kept in brown paper packets were brought to the laboratory and examined for the presence of asexual fruit bodies, acervuli. Experimental studies like isolation, purification culture, micro-photography, identification, pathogenicity testing of the isolated pathogens *etc.* were conducted following standard protocol under laboratory condition of the University, B.C.K.V. The purification of the isolated pathogen was carried out on PDA (Potato Dextrose Agar) medium but the fungus failed to produce acervuli on the medium. Thus after further studies using different media combinations it was identified that PAM (Peptone agar medium) was the ideal medium for acervuli production and sporulation of the isolated pathogen. Series of slides were prepared from culture or infected parts for morpho-metric studies of fungal spores, spore bearing and other structures. Micro-photograph of all fungal structures were taken with help of Compound microscope or Karl Zeis Phase Contrast Microscope (under 10x, 20x, 40x &100 x) and by using Canon Powers Shot A640 camera. Dimensions (*e.g.* length and breadth) of conidia, acervuli and hyphae of fungi were measured using AxioVision (Rel. 4.8) software. For pathogenicity establishment detached healthy leaves after proper cleaning with sterile distilled water and absolute alcohol, were pin pricked and artificially inoculated with fungal mat while pin pricked uninoculated (only agar bit) leaves were used as control. These were covered with transparent polythene packets for 48 hours and observed regularly till symptom development. The pathogen was re-isolated from the inoculated diseased parts of leaf and compared with the fungal culture isolated initially from

diseased leaf.

Results and Discussion

Anthracnose disease of *Aglaonema* occurs regularly in garden houses which ultimately reduce its marketable value. Though the disease occurs throughout the year in different ornamental growing areas, but severity and sporulation of the pathogen basically starts from May and continues up to November to end of January. Affected leaf samples were collected from the garden house during 2nd week of January, 2015. It was observed that 26 – 40% leaf area was covered with lesions. The severity of the foliage damage was 4 based on 0 – 6 scale.

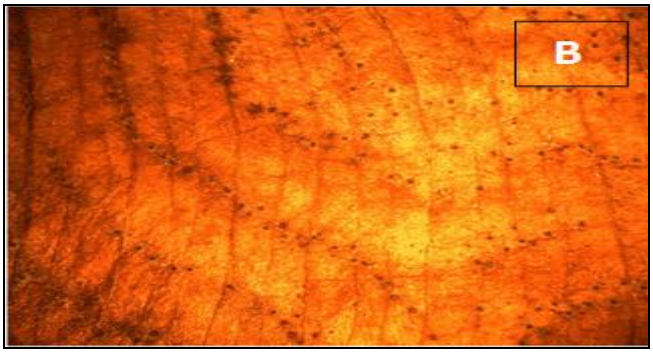
Symptoms of the anthracnose disease of *Aglaonema crispum*

The infection began as small, round to oval brown spots with grey centres, which generally appeared close to leaf margin. As disease progressed the leaves showed characteristic blighting like symptoms with light brown to grey coloured necrotic areas bordered by dark brown wavy margins. The spots might be sometimes surrounded by yellow halo. On the necrotic areas, black, erumpent, dot like acervuli appeared to be arranged in the form of concentric zonations on the upper surface of the leaf. Upon binocular observation, setae could be observed coming out of the host tissues.

Pathogenicity establishment

Pathogenicity of the pathogen was established by inoculating detached leaf under laboratory condition. The inoculated leaf produced same symptoms as observed in field. The pathogen was re-isolated from the inoculated diseased parts of leaf and compared with the fungal culture isolated initially from diseased leaf.

Plate 1a: Anthracnose symptom on the leaf of *Aglaonema crispum*



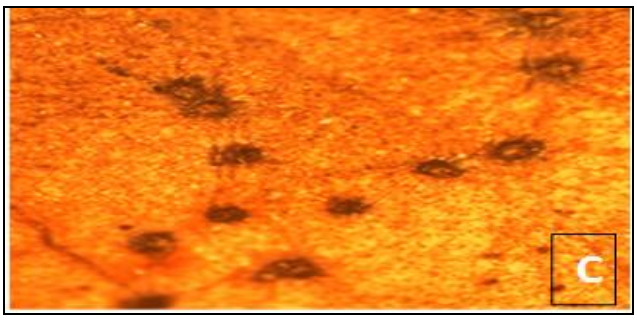


Plate 1 B, C: Binocular view of concentrically arranged acervuli (100 X)

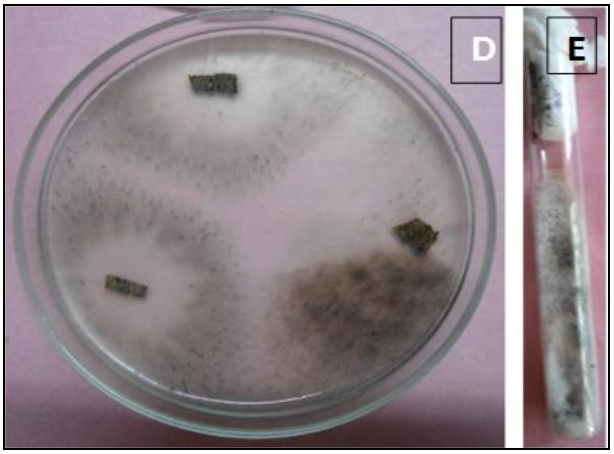
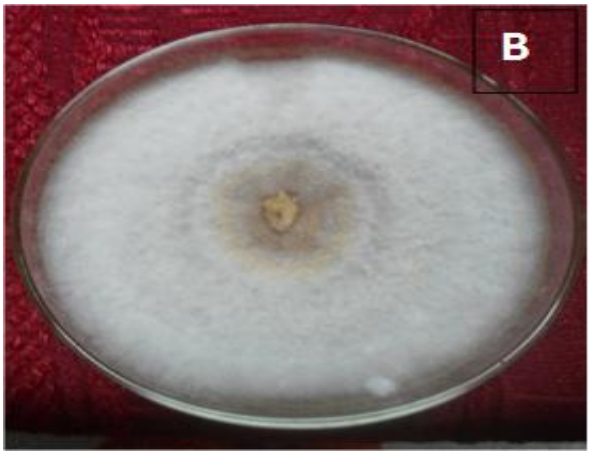


Plate 1 D, E: Isolation and purification of *Colletotrichum gloeosporioides* from infected leaf of *Aglaonema crispum*

Plate 1f: Pathogenicity establishment of *Colletotrichum* sp. of *Aglaonema crispum* under laboratory condition.

A. PDA medium



B. Peptone agar medium

Plate 2A, B: Cultural characteristics of *Colletotrichum* sp. of *Aglaonema crispum* on different media.

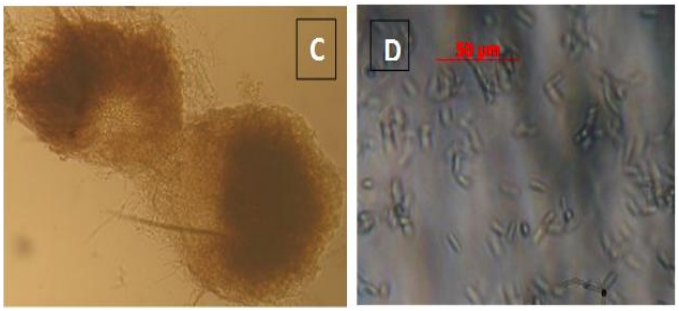


Plate 2 C, D: Microscopic view of avervuli bearing setae and conidia produced on *Aglaonema crispum*

Plate 2 E: Microscopic view of avervulus bearing setae and conidia produced on peptone agar medium

Cultural characteristics of the fungus observed on various media:

On PDA medium the fungus produced cottony white mycelial growth which was slightly convex and dense. Upon observation from below greyish coloured mycelia could be observed. Fluffy mycelial growth covered the media completely without any productions of fruiting bodies.

On peptone agar medium, mycelial growth was limited, hyaline to white colored. Acervuli were produced in linear manner which were initially hyaline but turned to black finally. The acervuli were numerous near the inoculated disc.

Morpho-metrical descriptions of various structures of the pathogen obtained from *Aglaonema crispum* and on PAM:

On the host black, dot like, numerous, superficial, erumpent acervuli were produced which were 320.7 – 540.1(av. 399) µ wide and concentrically arranged on leaf tips. Setae were numerous, black, 1 - 2 septate, unbranched, 126.0 – 176.3 (av. 148.8) x 3.5 – 6.8 (av. 5.2) µ in size with pointed tips. Conidia were hyaline, single celled, numerous, cylindrical to short rod shaped with rounded ends and 18.5 – 24.8 (av. 22.6) x 2.7 – 4.4 (av. 3.2) µ in size.

On the PAM (Peptone agar medium), the hyphae produced were hyaline, thin and septate. Its diameter varied from 10.3 – 14.8 (av. 13.1) µ. Acervuli were pale brown to black, 426.5 - 920.5 (av.628.9) µ in size. Size of acervuli varied greatly in the peptone agar medium. Setae were numerous, black to dark brown, 1 – 3 septate, unbranched, 198.5 – 245.1 (av. 221.0) x 6.0 – 7.8 (av. 7.0) µ in size with pointed tips. Conidia were hyaline, 1-celled, smooth walled, eguttulate, cylindrical with rounded ends measuring 23.7– 28.5 (av. 26.1) x 3.0 – 5.0 (av. 4.1) µ in size.

Discussion

Aglaonema spp. were known to be attacked by *Gloeosporium graffi* and *Colletotrichum dematium*. *Gloeosporium graffi* produced conidia which were hyaline, 1-celled, smooth, ellipsoid, guttulate, 11 - 13.5 µ x 3.5µ. It was known to attack *A. simplex* also. (c.f Sohi, 1990). *Colletotrichum dematium* produced colonies extremely variable, with white to pale mouse grey or pale grey vinaceous patches at the centre or elsewhere, reverse dark brown, conidial masses olive grey to light vinaceous salmon. Sclerotia were abundant, black and conical. Setae were abundant. Conidia were falcate, fusiform, apical acute, 19.5 - 24 x 2 - 2.5(3.5) µ. Appressoria were abundant, medium brown, clavate to circular, edge usually entire, 8 – 11.5 x 6.5 - 8µ, often becoming complex and forming long, closely branched chain (Sutton, 1980). Then the above mentioned description of our isolated pathogen was also verified with the description of *Colletotrichum gloeosporioides* given by Saccardo (1884). He described that conidiomata were acervulus, amphigenous, mostly epiphyllous, subepidermal. Setae were often present on acervuli but sometimes arising alone from stomata, forming dense fascicles and bearing enteroblastic conidia apically. Conidiogenous cells were discrete, enteroblastic, phialidic, hyaline and smooth. Conidia were slimy, formed singly, cylindrical, (10 -) 15 – 20 (- 25) x (3 -) 4 - 6 µ in size, apex obtuse, base sub-acute, aseptate, guttulate, hyaline, smooth, forming septum before germination. Appressoria with entire or sometimes slightly irregularly lobate margin were ovate, globose or ampulliform, brown to medium brown, 8 - 12 x 6 - 9 µ in size. When the acervuli and conidial characteristics of causal fungal pathogen of the present study were compared with both the above mentioned anthracnose causing fungal spp. it differed from *Gloeosporium* due to the presence of setae and also from *Colletotrichum dematium* due dissimilarity in spore shape and size but exhibit gross similarity with *Colletotrichum gloeosporioides*. So, the causal fungus of presently described anthracnose disease of *Aglaonema crispum* is being proposed as *Colletotrichum gloeosporioides*. It was the first record of the pathogen from West Bengal.

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